miRNAs belong to a class of small non-coding RNAs which can modulate gene expression. Disturbances in their expression and function may cause cancer formation, progression and cell response to various types of stress. The let-7 family is one of the most studied groups of miRNAs. The family contains 13 members with similar sequences and a wide spectrum of target genes. In this paper, we mostly focus on one member of the family – let-7d. This miRNA is dysregulated in many types of cancers. It can be over- or down-expressed, and it acts as a tumor suppressor or oncogene. It regulates various genes such as LIN28, C-MYC, K-RAS, HMGA2 and IMP-1. Moreover, let-7d has a significant impact on epithelial-to-mesenchymal transition (EMT) and formation of cancer initiating cells which are resistant to irradiation and chemical exposure and responsible for cancer metastasis. Let-7d can serve as a prognostic and predictive marker for personalization of the treatment. Let-7d is a small RNA with great power, but in different cell genetic backgrounds it acts in different ways, which makes this molecule still mysterious.

Key words: let-7d, let-7 family, miRNA, gene regulation, cancer, radiotherapy, chemotherapy.

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Review paper

The mystery of let-7d – a small RNA with great power

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MicroRNAs

miRNAs are a group of short, about 22 nucleotides long, non-coding RNAs. miRNA genes are situated within the introns or exons of coding genes or in intergenic regions [1]. They may possess their own promoters or share them with mRNA translation promoters. Some miRNAs are encoded as a single miRNA, others as multiple miRNAs creating clusters [2]. miRNA genes are transcribed from the genome by RNA polymerase II or III. The biogenesis of miRNAs is still not clear, but generally two different pathways of miRNA biogenesis are distinguished: "canonical" and "non-canonical". The canonical one is a two-stage process occurring in the nucleus and cytoplasm, where immature forms of miRNA hairpins are transformed to about 22 nt duplexes by Drosha and Dicer enzymes. One of the duplex strands, called the guide strand, is incorporated into the RISC complex, and miRNA in this complex can bind with the 3'UTR region of target mRNA [3–9]. Some data suggest that the second strand, miRNA*, can also take part in the regulation of gene expression [10, 11].

Regulation of gene expression is the function of miRNAs and is connected with two mechanisms of blocking protein translation: i) repression of mRNA translation or ii) cleavage of mRNA. miRNAs can regulate from 30% to 60% of human genes [12]. These small RNAs are associated with the cell cycle, apoptosis, proliferation, differentiation, metabolic pathways and cell response to various types of stress [13–18]. The relationship of miRNAs between important cellular processes and disturbance of miRNA expression, biogenesis, and function in cancer makes these small molecules one of the most studied nowadays.

Let-7d

The human lethal-7 (let-7) family plays a critical role in regulation of development and carcinogenesis. The family contains 13 members located in 9 different loci on chromosomes 3, 9-12, 19, 21, 22 and X (Fig. 1A) [19]. Let-7 miRNAs are the most abundant among all miRNAs [19]. They are conserved across species and are considered as an ancient miRNA [120]. They are located individually or as clusters, which can contain only let-7 members or also other miRNAs. Clusters are the result of vertebrate-specific genome duplications [20], which enable correct biogenesis of miRNAs and also precise regulation of them [2]. All members of the family are highly sequence-similar and share a common nucleotide motif named the "seed region", which is a crucial component for target recognition by RISC [4–6].

Let-7d is one of the members of this family. It is situated within the let-7a-1/let-7f-1/let-7d cluster, which is located in the human genome in region

B on chromosome 9q22.3. The cluster with a 10 kb upstream promoter encodes a single polycistronic transcript with 3 members, which constitute about 24% of all let-7 precursors [19]. The transcriptional activity of the promoter is nearly as strong as SV40. The cluster contains two MYC-binding sites. Binding of MYC protein to non-canonical E-box 3 causes inhibition of transcription, whereas binding to canonical E-box 2 enhances this process (Fig. 1C). Binding to E-boxes depends on the cancerous or non-cancerous cell character and ratio of MYC and MAX in the nucleus [19].

The post-transcriptional regulation of let-7 miRNAs is carried out on LIN28 and LIN28B. LIN28 binds to preE elements of the miRNA transcript and blocks pri-let-7 processing by Drosha and pre-let-7 by Dicer. Furthermore, LIN28 can recruit a terminal uridylyl transferase which adds uridine to pre-miRNA and causes its decay. LIN28 is associated with aggressive forms of cancer and causes down-regulation of let-7 miRNAs, especially the let-7f precursor [19, 21, 22]. Let-7d may be similarly regulated by an androgen effect in prostate cancer [23] and by PDGF in glioblastoma and ovarian cancer [24]. It is possible that, like in nematode worms, regulation of let-7 is controlled by let-7 itself. Mature let-7 with its effector protein, Argonaute, as miRISC can bind to pri-let-7 [25, 26].

Let-7d targets

The mature let-7 family members are the most abundant among all miRNAs in the cell and they are regulated by different transcriptional and post-transcriptional mechanisms. The characteristic feature of miRNAs from one family is sharing an identical seed sequence (Fig. 1B), so different members of the let-7 family could possess overlapping targets. Moreover, nucleotide changes in the pre-let-7 precursor make its stem fully complementary, whereby it functions as siRNA [27, 28]. There is a lack of comprehensive studies describing targets for all let-7 members and explaining whether all members regulate the same genes. Here, we focus on let-7d, which has only a few experimentally proven targets.

Let-7 family members are direct and strong regulator of the RAS family. K-RAS, N-RAS and H-RAS mRNAs contain let-7 binding sites in 3'UTR sequences [29, 30]. Inhibition of K-RAS mRNA by let-7d causes greater accumulation of cells in G1 than in G2/M phase of the cell cycle whereby cell proliferation is reduced [30].

The second known target is MYC [29]. There is a double-negative feedback loop between MYC and let-7 miRNAs. MYC expression is inhibited by let-7d, whereas MYC inhibits some members of the let-7 family. Moreover, the inhibitory function of MYC is shared with LIN28, which is involved in induced pluripotent stem cell (iPS) and tumor initiating cell (TIC) formation [19, 21, 29].

IMP-1 is also a target of the let-7 family. There is a connection among let-7, IMP-1 and MYC. Let-7 can reduce MYC protein expression directly by binding to its 3'UTR or indirect by depletion of IMP-1, which in turn destabilizes MYC mRNA. IMP-1 also regulates cell-cycle CDC25A and CDK6 [29, 31].

The next target of let-7 is HMGA2. This oncogene can skip let-7 regulation by loss of its let-7 binding sites, causing over-expression of this protein and leading to tumor formation [29]. Moreover, HMGA2 mRNA in some cases is a more sensitive target of let-7 than RAS [32].

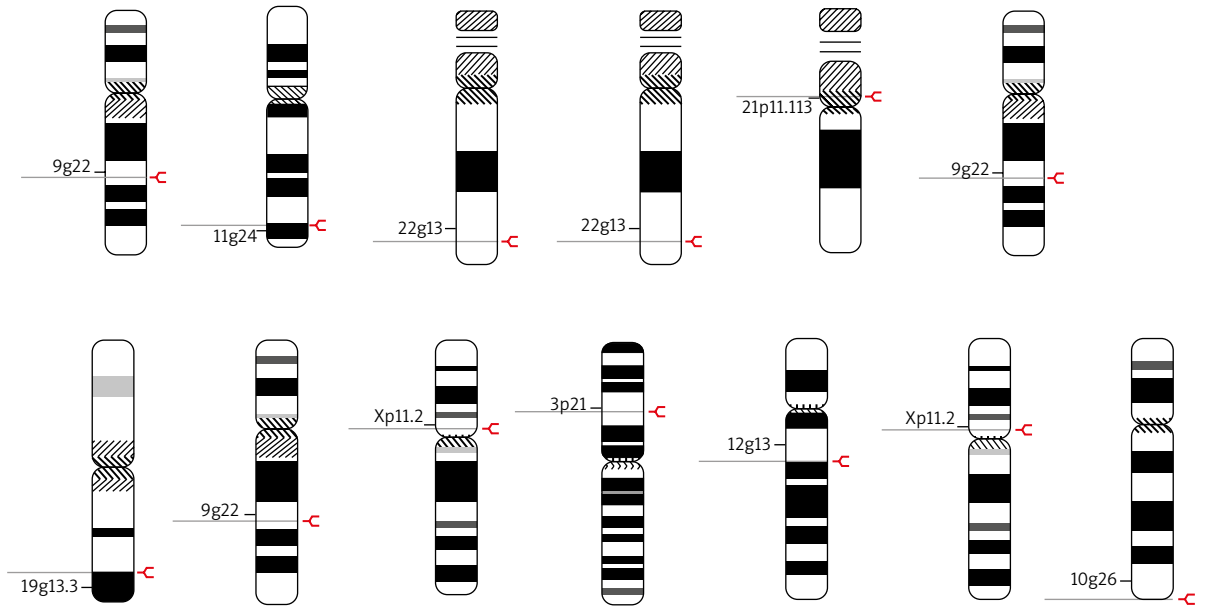
The group of genes regulated by let-7d also includes cell-cycle genes such as CDC25A, CDK6 and cyclin D1 [29]. The microarray analysis of gene expression in cells with let-7b over-expression indicates down-regulation of genes associated with cell cycle and division (cyclins, cell division cycle proteins, kinase-associated proteins) as well as genes coding for DNA synthesis and DNA replication. Surprisingly, let-7b causes inhibition of expression of both proven and putative tumor suppressor genes and the cell cycle checkpoint genes. On the other hand, let-7b up-regulates some genes such as the CDK inhibitor B2, MAX and cyclin G2, so it can act as a tumor suppressor [33]. Let-7c and let-7g regulate Bcl-$_{XL}$ – an anti-apoptotic member of the Bcl family [34]. Let-7d is presumable engaged in Dicer protein regulation and lack of it causes over-expression of this enzyme and proliferation of cancer cells in the oral cavity [35]. PBX3 [23], DMT1 [36] and caspase-3 [37, 38] are also indicated as let-7d targets. The long list of let-7 family targets, their functions and relation with cancer have been reviewed by Barh [39].

Taking into consideration only the seed region, let-7d should regulate the same targets as other family members. However, emerging data suggest that the let-7 family contains miRNAs with different activities [28]. Some evidence suggests that miRNAs from the same precursor target different genes, which are involved in different cellular processes [2]. The gene targeting is made more complicated by the existence of 3' and 5' variants of one miRNA (isomiRs) created by Drosha and/or Dicer enzymes. The 3' end of miRNAs can be modified by exoribonucleases as well as nucleotidyltransferases, and RNA editing can cause modification of the "seed region". All of these events produce different variants of one mature miRNA, which could have different targeting properties [2, 40]. The inhibition of proteins depends largely on the cell genetic background, and let-7 members might cause different degrees of translation inhibition and mRNA instability depending on target genes [32, 33]. Moreover, some miRNAs probably can not only inhibit but also up-regulate protein levels. Let-7 belongs to the group of these miRNAs [41]. There is still a lack of precise knowledge about let-7 targets as well as full understanding of let-7 family members function. Some of the targets of let-7d predicted by TarBase (DIANA Tools) and miRDB have been shown in Table 1A and for let-7d* in Table 1B. The analysis reveals over 300 target genes for let-7d-5p and 40 for let-7d-3p.

Let-7d and cancer

Half of the known miRNA genes are located close to or inside chromosomes regions, which are usually mutated in cancer, known as fragile sites and cancer-associated genome regions [42, 43]. Single miRNA can function in cancer as a tumor suppressor or oncogene (oncomiR), or have dual function. Down-regulation of suppressor miRNAs and

A



B

miRNA	miRBase number	Sequence
hsa-let-7a-1	MI0000060	GGGA**UGAGGUAGUAGGUUGUAUAGUU**
hsa-let-7a-2	MI0000061	AGGU**UGAGGUAGUAGGUUGUAUAGUU**
hsa-let-7a-3	MI0000062	**UGAGGUAGUAGGUUGUAUAGUU**
hsa-let-7b	MI0000063	**UGAGGUAGUAGGUUGU**G**U**G**GUU**
hsa-let-7c	MI0000064	**UGAGGUAGUAGGUUGUAU**G**GUU**
hsa-let-7d	MI0000065	A**GAGGUAGUAGGUUGCAUAGUU**
hsa-let-7e	MI0000066	**UGAGGUAG**G**AGGUUGUAUAGUU**
hsa-let-7f-1	MI0000067	UCAGAG**UGAGGUAGUAG**A**UUGUAUAGUU**
hsa-let-7f-2	MI0000068	GUCGGA**UGAGGUAGUAG**A**UUGUAUAGUU**
hsa-let-7g	MI0000433	**UGAGGUAGUAG**U**UUGUA**C**AGUU**
hsa-let-7i	MI0000434	**UGAGGUAGUAG**U**UUGU**GCU**GUU**
hsa-mir-98	MI0000100	**UGAGGUAGUA**A**GUUGUAU**U**GUU**
hsa-mir-202	MI0000130	**U**UCCUAU**G**C**A**UA**U**AC**U**UCUU**U**G

C

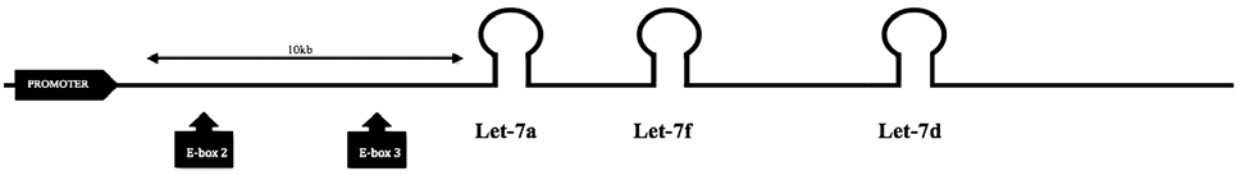


Fig. 1. Characterization of let-7 family: **A)** genomic localization of its members; arrows show exact localization of miRNA within chromosome; **B)** sequence similarity and **C)** structure of let-7a-1/let-7f-1/let-7d cluster. Data obtained from GenBank (NCBI) and miRBase databases

up-regulation of oncomiRs is linked to initiation of proliferation, invasion, angiogenesis and metastasis of tumor [44–49].

Let-7d is involved in regulation of many important genes, as described in the previous section, so it is not surprising that it has a significant role in cancer. It is believed that members of the let-7 family act as tumor suppressor miRNAs and regulate expression of many oncogenes by both direct and indirect pathways [28]. Expression levels of let-7 family members are significantly low in human cancers, but in some cases high expression levels are indicated [39]. The mechanism of this phenomenon is still not clear, but it is probably connected with complex let-7 family transcription and post-transcription regulations, genes' copy number of let-7 members and epigenetic modulations [50, 51]. It has been proven that deletion of some let-7 miRNAs or let-7 clusters is cancer-type dependent [50].

The expression of let-7d is deregulated in cancers such as pancreatic, prostate, primary pigmented nodular adrenal dysplasia, head and neck, ovarian, breast, bladder, kidney and retinoblastoma [28, 39, 52–54].

The implications of the let-7 family in cancer are multifaceted due to regulation of the cell cycle, proliferation, and apoptosis pathways, as described above. Moreover, the let-7 family has an influence on differentiation, epithelial-to-mesenchymal transition (EMT) and TIC formation [55–57].

Over-expression of let-7d causes cell differentiation and changes in cell phenotype *in vitro*. For example, neural stem cells transfected by let-7d differentiated into astroglial cells. The proliferation of neural stem cells has been suppressed and cell migration in mouse brain has been observed. Let-7d regulates these effects on neurogenesis by modulating miR-9 and TLX [58]. The other studies showed, that fibroblasts are less mesenchymal-like and more similar to epithelial cells. In this case let-7d partly changed cell phenotype, probably by affecting HMGA2, SLUG, ID1 and ID2, but did not influence TWIST and SNAIL [59, 60]. On the other hand, inhibition of let-7d upregulates HMGA2 and some markers characteristic for mesenchymal cells. Moreover, let-7d seems to be under direct transcriptional regulation of transforming growth factor β (TGF-β) [61]. Let-7d functions as a switch between EMT and MET (mesenchymal-epithelial transition) processes and regulates the TIC cell population.

The TICs are a small fraction of tumor cells displaying the capacity for self-renewal and differentiation into new cancer cells. Highly tumorigenic behavior and radio- and chemoresistance are their features. The let-7 family is down-regulated by post-transcriptional mechanisms in TICs and up-regulated during the differentiation process. In the group of let-7 oncofetal genes suppressed in most adult tissues but activated in various forms of cancer are HMGA2 (promotes self-renewal of stem cells), IMP-1 (stabilizes some RNAs such as C-MYC and protects them from degradation), LIN28/LIN28B (responsible for pluripotency), RAS and MYC [56]. Down-regulation of let-7d in head and neck squamous cell carcinoma lines activated TWIST and SNAIL expression, whereas up-regulation of let-7d reversed the phenotype. Modulation of let-7d changed the cell nature and controlled ALDH+/– cell populations, where ALDH+ cells are described as TICs [55].

It is proven that the let-7 family has a significant role in cancer biology, but it is not explained whether disturbance of let-7 miRNA expression causes cancer transformation or these disturbances are caused by cancerous changes in the cell. It is also not clear why, in some cancer, expression of let-7 members is down- or up-regulated. Some independent studies regarding the same cancer type indicated both possibilities: for example, let-7d is indicated as up- as well as down-regulated in prostate cancer [62]. Sometimes let-7 profiling is much more complicated, because in the same cancer patient group, cases with low, unchanged and high let-7 expression can be found [62]. It is accepted that let-7 miRNAs are suppressors, and this statement is supported by many observations and experimental studies. However, we can also find evidence for the oncogenic role of some let-7s as a result of targeting caspase-3 [37, 38] and BAX mRNAs [63, 64]. Is it possible to definitely say what is the exact role of let-7 family members in cancer: suppressor or oncogene or maybe both of them? What affects the role of let-7 miRNAs in cancer cells? These questions are still open.

Let-7d and response to irradiation and chemical exposure

Studies have shown dual behavior of the let-7 family after irradiation – miRNAs of this family can be up- or down-regulated [65, 66]. The behavior of let-7 members (like other miRNAs) depends on dose, time after irradiation, source of oxidative stress and genetic background of the cell [67-69]. For example, in two glioblastoma cell lines, with different DNA-PK activity, that let-7 members (including let-7d) are mostly up-regulated in that cell line which is more radioresistant [67]. In contrast, in lung cancer cell lines let-7 members (including let-7d) are mostly down-regulated [54].

The let-7 members regulate C-MYC and RAS expression. It has been shown that C-MYC or N-RAS alone increases radiation sensitivity, whereas together they increase radioresistance [70]. Down-regulation of let-7 family members depends on the dose and may be regulated directly by p53 as well as indirectly by ATM protein. The let-7 family seems to be a critical factor for the cellular response to oxidative stress and is potentially involved in protection against radiation cytotoxicity. In contrast, over-expression of let-7a causes decreased K-RAS expression and radiosensitization of cells. The RAS protein is regulated through the Lin28-let-7 network [71]. The let-7 family's impact on radiosensitivity has been confirmed *in vitro* [72]. Over-expression of let-7 affects the RAS oncogene and genes associated with DNA damage repair: RAD51, RAD21, FANCD2 and CDC25 [72]. Different patterns of miRNA expression have been shown in irradiated and bystander cells. The let-7 family is up-regulated in irradiated cells as opposed to bystander cells, in which most of these miRNAs are repressed [73]. The changes in miRNA expression can be observed just a few minutes after irradiation. Some authors report that peaks of up- or down-regulation are found at 4 hours after irradiation, at the time of the most active DNA repair processes. Most of the miRNAs return to their baseline levels after 24 hours [68, 69, 74]. Changes of miRNAs

Table 1. Predicted target genes for: **A)** let-7d-5p and **B)** for let-7d-3p (let-7d*). Let-7d-5p has over 300 and let-7d-3p has 40 predicted target genes. Some of them have been experimentally validated. Data obtained from miRDB and TarBase databases. Target scores equal to and over 0.8 (for A) and 0.7 (for B) have been choosen as criteria for let-7d targets; *target score taken from TarBase

A

Target score	Validated by	Gene symbol	Gene description
0.96/0.99*	sequencing	LIN28B	lin-28 homolog B (C. elegans)
0.971*	sequencing	ZNF280B	zinc finger protein 280B
0.969*	sequencing	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
0.965*	sequencing	TMEM135	transmembrane protein 135
0.955*	sequencing	IGF1R	insulin-like growth factor 1 receptor
0.951*	sequencing	KPNA5	karyopherin alpha 5 (importin alpha 6)
0.939*	sequencing	HAND1	heart and neural crest derivates expressed 1
0.939*	sequencing	TGFBR1	transforming growth factor, beta receptor 1
0.931*	sequencing	SMARCAD1	SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing DEAD/H box 1
0.922*	sequencing	USP24	ubiquitin specific peptidase 24
0.920		LRIG3	leucine-rich repeats and immunoglobulin-like domains 3
0.920		DDI2	DNA-damage inducible 1 homolog 2 (S. cerevisiae)
0.911*	sequencing	ONECUT2	one cut homebox 2
0.896*	sequencing	SLC10A7	solute carrier family 10, member 7
0.89/1.00*	sequencing	IGF2BP1	insulin-like growth factor 2 mRNA binding protein 1
0.880*	sequencing	ARID3A	AT rich interactive domain 3A
0.880		PRTG	protogenin
0.872*	sequencing	SPRYD4	SPRY domain containing 4
0.870	expression observation	HMGA2	high mobility group AT-book 2
0.869*	sequencing	FAM104A	family with sequence similiarity 104, member A
0.850		NAP1L1	nucleosome assembly protein 1-like 1
0.850		USP38	ubiquitin specific peptidase 38
0.850		COIL	coilin
0.847*	sequencing	C11orf57	chromosome 11 open reading frame 57
0.842*	sequencing	ZNF644	zinc finger protein 644
0.840		LIMD2	LIM domain containing 2
0.840		ADRB2	adrenergic, beta-2 receptor, surface
0.830		DCLRE1B	DNA cross-link repair 1B
0.830		GATM	glycine amidinotransferase (L-arginine: glycine amidinotranserase)
0.830		FIGNL2	fidgetin-like 2
0.830*		IGDCC4	immunoglobulin superfamily, DCC subclass, member 4
0.822*		GLMN	glomulin, FKBP associated protein
0.820		COL14A1	collagen, type XIV, alpha 1
0.820		DMD	dystrophin
0.815*	sequencing	NHLRC3	NHL repeat containing 3
0.810		SLC5A9	solute carrier family 5 (sodium/glucose cotransporter), member 9
0.800		GDF6	growth differentiation factor 6
0.800		MAP4K3	mitogen-activated protein kinase 3

B

Target score	Validated by	Gene symbol	Gene description
0.840		MEX3C	mex-3 homolog C (C. elegans)
0.810		NOM1	nucleolar protein with MIF4G domain 1
0.760		SH3RF1	SH3 domain containing ring finger 1
0.730		PTAR1	protein prenyltransferase alpha subunit repeat containing 1
0.730		KIAA1267	KIAA1267
0.710		PARP11	poly (ADP-ribose) polymerase family, member 11

after irradiation can be a result of cell protection causing increased expression of genes responsible for DNA repair and decreased levels of pro-apoptotic genes. However, it is possible that miRNAs in irradiated cells tend to return to levels of genes changed after stress. Nonetheless, the exact regulatory mechanisms and role of miRNAs in response to irradiation still remain unclear.

Loss of let-7 family function is associated with drug resistance in various cancers. The known mechanisms are both direct and indirect. The direct influence of let-7c and let-7g is based on targeting 3'UTR of Bcl-$_{XL}$, which leads to its decrease in human hepatocellular carcinoma. Up-regulation of let-7 sensitizes cells to sorafenib by targeting MCI-1, a member of the Bcl-2 anti-apoptotic family [34]. Another direct effect of let-7 (let-7a and let-7d) is connected with inhibition of caspase-3. Two independent studies reported that let-7 has a complementary seed sequence to 3'UTR of caspase-3 mRNA [37, 38]. The regulation by let-7 refers to the caspase-3 only but not to caspase-8 or -9. In this case, over-expression of let-7a reduces sensitivity of cells to agents such as doxorubicin, paclitaxel or interferon γ. In this context, let-7 acts as an oncomiR instead of a suppressor miRNA [38]. The high let-7a expression and paclitaxel treatment may together stimulate expression of IGF-II. High expression of IGF-II causes progression of ovarian cancer. However, let-7a may synergistically interact with platinum drugs by inhibition of DNA repair systems such as BRCA1. In this context, high expression of let-7a supports the platinum effect [75].

The indirect influence of let-7g via IMP1 stabilizes mRNA of MDR-1. The MDR-1 (ABCB1) gene, a member of ATP binding cassette transporters (ABC transporter family), encodes the membrane transporter P-glycoprotein responsible for multidrug resistance [76]. The regulator of miRNA biogenesis LIN28 is up-regulated in breast cancer cells which are resistant to paclitaxel. As mentioned above, LIN28 causes down-regulation of let-7a and let-7b and induces expression of p21 and RB. There is a feedback loop between let-7 and LIN28 regulating cell phenotype. Over-expression of LIN28 is characteristic for TICs, local relapse and metastasis of cancer. Moreover, the expression of LIN28 dramatically increases in cancer tissue after neoadjuvant chemotherapy. Restoring let-7 expression increases sensitivity to paclitaxel [77]. In head and neck cancers, over-expression of let-7d and let-7a reduces chemoresistance by depletion of TICs with ALDH+ phenotype and sensitizes to cisplatin and 5-FU [55, 78].

In sum, there is limited knowledge about the role of let-7d in the cell response to radiation and chemotherapeutic drugs. The exact function is not determined, and potentially let-7d may function like other members of the let-7 family by targeting the same pathways. Experimental studies should dispel doubts about this phenomenon.

Let-7d in diagnostics and treatment

The members of the let-7 family are connected with many features of cancer and could be applied as diagnostic, predictive and prognostic biomarkers.

Let-7d alone or together with other genes may be used for cancer profiling and serve as a diagnostic marker. The cancers can be divided into two groups: let-7^{high}- and let-7^{low}-expressing. The let-7^{high} cancers are more differentiated and display an epithelial phenotype [79]. But in contrast, let-7d is up-regulated in invasive ductal carcinoma [80]. These data suggest that the level of let-7 expression is not a universal marker of tumor aggressiveness. Even so, changes in let-7 still seem to be a marker of cancer transformation and progression [81] and enable cancer to be distinguished from normal tissue and different pathological types of tumor [82]. The use of miRNA seems to be a more sensitive tool than the currently used histopathological methods.

It has been shown that modification of let-7d level caused changes in cell line resistance to cisplatin and 5-FU [55]. There is no clinical study about let-7d's influence on chemotherapy. However, analysis of let-7a levels in ovarian cancer patients can be used as a predictive marker. The patients with high expression of let-7a respond better to platinum treatment. Combined treatment, platinum with paclitaxel, is more beneficial for patients with low let-7a expression [75].

A combined low level of let-7d and miR-205 is a poor prognostic marker in head and neck cancer patients, and it seems to be independent of anatomical site, tumor size, treatment and HPV status [83]. Similarly, ovarian cancer patients with a low combined score of HMGA2 and let-7d have better prognosis than the group with a high HMGA2/let-7d ratio [79]. Likewise, assessment of let-7d* expression can be used as a predictor of recurrence risk for hepatocellular carcinoma [84]. In contrast to this, pancreatic cancer patients with a high level of let-7d in plasma have the worst prognosis, but the authors of the study normalized the results to miR-16 [85], which is a poor normalizing factor [88].

The diagnostics tends to use biomarkers which can be simply achieved from the patients at any time during treatment. Circulating miRNAs from whole blood or serum can be used as non-invasive biomarkers for hematological malignancies and solid tumor detection [86, 87]. However, one of the emerging problems of circulating miRNAs is their normalization. The use of a combination of let-7d/let-7g/let-7i as normalization control for circulating miRNAs is supposed to be a more reliable solution than commonly used reference genes [88].

Restoration of a member or members of the let-7 family in cancer cells is a new promising gene therapy. Restoration of let-7d should inhibit cancer proliferation and metastasis, deplete TICs and sensitize to chemo- and radiotherapy. The delivery of anti- or miRNAs is based on viral and non-viral vehicles [89–93]. Some preclinical and clinical studies with let-7 members are currently in progress [94–96]. The application of miRNAs in cancer therapy may prove to be superior to siRNAs or shRNAs because interfering miRNA (miRNAi) modifies overlapping targets containing complementary regions to the seed sequence of miRNA in a natural way [97]. The effect is mild and simultaneous on a number of oncogenes and pathways [50]. The introduction of artificial miRNAs may be less toxic than traditional chemotherapy. The eventual off-target effect and toxicity caused by miRNA are mild or negligible [98, 99].

Summary

Even though miRNAs are among the most analyzed molecules nowadays, the let-7 family still seems to be mysterious. The family contains 13 members with almost identical sequences and ability to regulate different targets. Moreover, one let-7 member regulates various genes. The behavior of let-7 members can depend on their activity, genetic cell context or tumor microenvironment. They are deregulated in various cancers by different mechanisms, and their function is not fully defined. The members may display different functions in the same cell and sometimes can behave as a suppressor or an oncogene. Many studies have shown that the let-7 family is implicated in proliferation, invasion, angiogenesis and tumor metastasis, can change cell phenotype through the EMT process, and regulates TIC populations. Multifaceted let-7 modulates cancer response to radio- and chemotherapy. However, there is a lack of comprehensive studies about all members. Knowledge about the role of individual members, such as let-7d, is based on assumptions and comparisons to other members of the family.

The potential of the family probably can be used for early cancer diagnostics, prediction, treatment personalization and new therapeutic miRNA technology in the future, but firstly, we should reveal the secrets about all members of the let-7 family.

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